sequence of SEQ ID NO:12; and the recombinant DNA from PCR E, a part of the nucleotide sequence of SEQ ID NO:11. Restriction enzyme digestion was applied to the recombinant DNA from PCR C with *Nde*I and *Bam*HI, the recombinant DNA from PCR E with *Bam*HI, and plasmid vector "pET-3a", employed in Example 1-3(a), with *Bam*HI. Appropriate amounts of these digests were placed in a micro-reaction tube and subjected to ligation reaction using "LIGATION KIT VERSION 2", commercialized by TAKARA SHUZO Co., Ltd., Ohtsu, Shiga, Japan, in accordance with the accompanying instructions. By conventional methods, transformation of competent cells of *Escherichia coli* strain "JM109", commercialized by TAKARA SHUZO Co., Ltd., Ohtsu, Shiga, Japan, with the ligation product, cultivation of the transformants, and collection of a recombinant DNA from the cultures were carried out. Analysis by dideoxy method confirmed that the recombinant DNA contains the nucleotide sequence of SEQ ID NO:20 coding for the amino acid sequence of SEQ ID NO:10 and named "pEscFv#125-2H.HT". The amino acid sequence of SEQ ID NO:10 consists of the amino acid sequences of SEQ ID NO:2 for the heavy chain variable region in the monoclonal antibody "#125-2HmAb", a linker composed of glycine and serine, a part of SEQ ID NO:1 for the light chain variable region in the antibody, and six residues of histidine, which are positioned in this order from the N-terminus. As shown in FIG.4, the recombinant DNA "pEscFv#125-2H" orderly contained an initiation codon, the nucleotide sequence of SEQ ID NO:20, and a termination codon downstream of T7 promotor and ribosome binding sequence. Competent cells of *Escherichia coli* strain "BL21(DE3)pLysS", employed in Example 1-3(a), were transformed in usual manner with

the recombinant DNA "pEscFv#125-2H.HT" to obtain a transformant. Thus-obtained transformant was named "EscFv#125-2H.HT".

Example 2-2

Production of peptide by transformant

The transformant "EscFv#125-2H.HT", obtained in Example 2-1, was cultivated correspondingly to Example 1-3(b) in a 100ml scale. Collection of cells from the culture, collection of the inclusion body fraction after disrupting the cells, and wash of the inclusion body fraction were carried out similarly as in Example 1-3(b) to obtain the washed inclusion body fraction. To the washed inclusion body fraction, 10% volume of 0.1M Tris-HCl buffer (pH7.0) containing 6M guanidine hydrochloride (hereinafter called "6M guanidine-HCl solution") was added and stirred at 4°C overnight to solubilize the inclusion bodies. The solubilization product was applied to a column of 5ml affinity chromatography gel "Ni-NTA agarose", commercialized by QIAGEN GmbH, Hilden, Germany, and through the column 6M guanidine-HCl solution and 25mM Tris-HCl buffer (pH7.0) containing 50mM imidazole and 6M urea were run in this order to remove non-adsorbed components. Then 25mM Tris-HCl buffer (pH7.0) containing 250mM imidazol and 6M urea was run through the column to elute and collect adsorbed components. The collected fraction was diluted with 50mM Tris-HCl buffer (pH7.0) containing 6M urea to give a protein concentration of less than 0.1mg/ml and then dialyzed at 4°C against 0.1M Tris-HCl buffer (pH7.0) containing 0.4M L-arginine-HCl and 2mM EDTA (hereinafter called "TAE buffer") to renature the proteinaceous components. After the dialysis was repeated thrice, dialysis was further conducted against TAE buffer containing 10mM oxidized glutathione at 4°C for six days.

The dialyzed product was concentrated by ultrafiltration and then dialyzed against PBS. Analysis by conventional SDS-PAGE revealed that the dialyzed product contained a peptide of about 29kDa in a purity of about 95% or higher. The dialyzed product was lyophilized, resulting in a solid containing about 1mg of the peptide.

The solid was dissolved in RPMI1640 medium supplemented with 10%(v/v) fetal calf serum to give desired various peptide concentrations for the test samples, which were then examined by the test in Example 1-1(a) for IL-18-neutralizing activity. The monoclonal antibody "#125-2HmAb" was also prepared similarly as in Example 1-1(b) and diluted to give desired various antibody concentrations with the same medium for the test samples, which were examined as above. After the test, IFN-γ amounts measured in the testing systems were calculated for percentages to that of control to estimate percent inhibition of the induction of IFN-γ by IL-18. The results are in FIG.5.

As shown in FIG.5, the peptide of this Example dose-dependently and effectively inhibited the IL-18 biological activity to induce IFN-γ production from KG-1 cells. The molecular weight of the peptide of this Example estimated by SDS-PAGE well coincided with the calculated molecular weight of the amino acid sequence of SEQ ID NO:10, about 29kDa. These results indicate that the peptide is a type of the peptide of this invention, having the amino acid sequence of SEQ ID NO:10, an artificially produced peptide which neutralizes IL-18 and contains a part or the whole of the amino acid sequences of SEQ ID NOs:1 and 2, of the variable regions in anti-IL-18 antibody. The results in FIG.5 also shows that the peptide of this Example

exhibited IL-18-neutralizing activity with nearly equivalent efficiency to the monoclonal antibody "#125-2HmAb" in about twice mol concentration of the antibody. While the antibody belongs to $IgG_1$ to have two antigen-binding sites per molecule, the peptide of this Example is considered to have one. The results, therefore, indicate that the peptide of this Example neutralizes IL-18 with nearly equivalent efficiency to the parental antibody, and that the amino acid sequences of SEQ ID NOs:1 and 2 are partly or wholly useful in artificial producing of IL-18-neutralizing peptides. These results also indicate that the DNA obtained in this Example is a type of the DNA of this invention, coding for the present peptide, and the DNA facilitates the production of the peptide by the process using the DNA. In addition, when examined similarly as in Example 1-3(d) for binding to IL-18, the peptide of this invention specifically bound to IL-18.

Example 3

Peptide and DNA coding for the peptide

A type of the peptide of this invention in the form of a chimeric antibody is produced as follows. A DNA containing the nucleotide sequence coding for the constant region on human immunoglobulin light chain (κ chain) is first isolated from human genomic library in accordance with the procedures by P. A. Hieter et al., in "Cell", Vol.22, pp.197-207 (1980). By conventional PCR using the isolated DNA as template, a DNA is prepared to substantially consist of the nucleotide sequence coding for the constant region, hereinafter called "human light chain constant region DNA". By PCR similarly as PCR A in Example 1-2, another DNA is prepared to have a sequence consisting of the nucleotides

1-384 of SEQ ID NO:27, hereinafter called "mouse light chain variable region DNA". Using the PCR-prepared DNAs as template, the method designated "overlap extension", described in Robert M. Horton, "Methods in Enzymology", Vol.217, pp.270-279 (1993), is conducted to prepare a DNA comprising the mouse light chain variable region DNA followed by the human light chain constant region DNA and restriction enzyme recognition sites positioned at the 5′- and 3′-termini. A DNA for an expression vector which contains, like as "pSV2-neo" (ATCC 37149), a replication origin in *Escherichia coli*, a promotor and/or enhancer functioning in a mammalian cell, restriction enzyme recognition sites in regulatable position thereby, selection sequences, etc., is then prepared. The expression vector and the above-prepared DNA comprising the human light chain constant region DNA and mouse light chain variable region DNA are subjected to restriction enzyme digestion followed by ligation using ligase to obtain a recombinant DNA containing a sequence coding for a chimeric antibody light chain.

A DNA containing the nucleotide sequence coding for the constant region on human immunoglobulin heavy chain (γ chain) is isolated from human genomic library in accordance with the procedures by N. Takahashi et al., in "Cell", Vol.29, pp.671-679 (1982). The isolated DNA comprises four independent exons as described in the paper. Using the isolated DNA as template, the above-mentioned "overlap extension" is conducted to prepare a DNA with the exons directly connected, hereinafter called "human heavy chain constant region DNA". By PCR similarly as PCR B in Example 1-2, another DNA is prepared to have a sequence consisting of the nucleotides 1-423 of SEQ ID NO:28, hereinafter

called "mouse heavy chain variable region DNA". Using the PCR-prepared DNAs as template, the above-mentioned "overlap extension" is conducted to prepare a DNA comprising the mouse heavy chain variable region DNA followed by the human heavy chain constant region DNA and restriction enzyme recognition sites positioned at the 5′- and 3′-termini. A DNA for an expression vector which contains, like as "pSV2-gpt" (ATCC 37145), a replication origin in *Escherichia coli*, a promotor and/or enhancer functioning in a mammalian cell, restriction enzyme recognition sites in regulatable position thereby, selection sequences, etc., is then prepared. The expression vector and the above-prepared DNA comprising the human light chain constant region DNA and mouse light chain variable region DNA are subjected to restriction enzyme digestion followed by ligation using ligase to obtain a recombinant DNA containing a sequence coding for a chimeric antibody heavy chain.

The recombinant DNAs containing the sequences for the chimeric antibody heavy and light chains are next co-introduced by electroporation into mammalian established cell line such as CHO-K1, ATCC CCL-61. The DNA-introduction product is screened on the basis of the selection sequences on the expression vectors, and the selected cells are independently cultivated. The culture supernatants are examined by the test in Example 1-1(a) for IL-18-neutralizing activity. Cells which produce the positive culture supernatants are subjected to limit dilution into a single cell to obtain a transformant which produces the peptide of this invention in the form of a chimeric antibody. The transformant is cultivated in larger scale, and the culture supernatant is subjected to conventional methods for antibody

purification to obtain the peptide, in the form of a chimeric antibody. The peptide thus obtained effectively neutralizes IL-18 similarly as the anti-IL-18 monoclonal antibody "#125-2HmAb". The DNA according to this Example can be changed in sequences for the framework structures to code for similar amino acid sequences to the case of an human antibody obtainable from conventional databases by homology search with the peptide of this Example, and the changed DNA can be expressed to obtain another type of the peptide in the form of a humanized antibody comprising human framework structures. The humanized antibody thus obtainable can be predicted on three dimensional structure based on the amino acid sequence using conventional computational programs for protein structure analysis, and the predicted structure can be compared with the structure of the monoclonal antibody "#125-2HmAb" similarly predictable. Then the DNA for the humanized antibody can be further changed to express a three dimensional structure more closely resembled to the monoclonal antibody "#125-2HmAb", leading to obtainment of a humanized antibody which can exhibits substantially equivalent functions to the parental monoclonal antibody, "#125-2HmAb". The peptide of this Example and the peptides in the form of a humanized antibody form obtainable therefrom are useful in the treatment of the susceptive diseases.

Example 4

Liquid Agent

Peptides were prepared in accordance with the methods in Examples 1 and 2. Either of the peptide was dissolved to give a concentration of 1mg/ml in physiological saline containing as stabilizer 1%(w/v) powdered trehalose crystals "TREHAOSE®",

commercialized by HAYASHIBARA Co., Ltd., Okayama, Japan, and sterilized in usual manner by membrane filtration to obtain a liquid agent.

The products are excellent in stability and useful in an injection, ophthalmic solution, collunarium, etc., to treat and prevent the susceptive diseases including autoimmune diseases.

Example 5

Dried injection

Peptides were prepared in accordance with the methods in Examples 1 and 2. One hundred milligrams of either of the peptide was dissolved in 100ml of physiological saline containing 1%(w/v) sucrose as stabilizer. The solution was sterilized in usual manner by membrane filtration and divided into aliquotes of 1ml per vial, which were lyophilized before sealing.

The products are excellent in stability and useful as a dried injection to treat and prevent the susceptive diseases including autoimmune diseases.

Example 6

Ointment

Carboxyvinylpolymers "HI-BIS-WAKO 104", commercialized by WAKO PURE CHEMICALS, Tokyo, Japan, and powdered trehalose crystals "TREHAOSE®", commercialized by HAYASHIBARA Co., Ltd., Okayama, Japan, were dissolved in sterilized distilled water to give respective concentrations of 1.4%(w/w) and 2.0%(w/w). The solution was mixed to homogeneity with either of peptides prepared in accordance with the methods in Examples 1 and 2 and adjusted to pH7.2 to obtain a paste containing 1mg of the present peptide per 1g.

The products are excellent in spreadability and stability

and useful as an ointment to treat and prevent the susceptive diseases including autoimmune diseases.

Example 7

Tablets

Powdered anhydrous α-maltose crystals "FINETOSE®", commercialized by HAYASHIBARA Co., Ltd., Okayama, Japan, was mixed to homogeneity with either of peptides prepared in accordance with the methods in Examples 1 and 2 and cell activating agent "LUMIN", [bis-4-(1-ethylquinoline)][γ-4'-(1-ethylquinoline)], and the resulting mixture was tabletted in usual manner to obtain tablets containing 1mg of the present peptide and 1mg of "LUMIN" per tablet.

The products, with swallowability, stability, and cell activating property, are useful as tablets to treat and prevent the susceptive diseases including autoimmune diseases.

Experiment

Acute Toxicity Test

Each agent in accordance with Examples 4-7 was administered in usual manner to 8-week-old mice through percutaneous, peroral, or intraperitoneal route. In any route, $LD_{50}$ of the tested samples were about 1mg/kg-body-weight or higher on the present peptide basis. These results support the safeness of the present peptide incorporated in pharmaceuticals directed to the uses for mammals including humans.

As explained above, this invention is based on artificially production of the peptides which effectively neutralize a biological activity of IL-18. The present peptide is efficacious in the alleviation of rejection reaction relating to grafting organs and the treatment and prevention of various diseases

caused by excessive immunoreactions because the peptide suppresses and regulates immunoreactions of mammals including humans. The inhibitor, inhibition method, neutralizer, and neutralization method of this invention, which use the present peptide, are effectively used to treat various diseases directly or indirectly involving IL-18 biological activities and to suppress rejection reaction and excessive immunoreactions caused by grafting organs. The present peptide with such usefulness is easily produced in desired amounts by the process of this invention. Furthermore, the present peptide is useful for a reagent to screen for agonists and antagonists to IL-18.

This invention exhibits these remarkable effects and greatly contributes to the art.

While there has been described what is at present considered to be the preferred embodiments of this invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.